Fig. 1 – Double Selection elongation

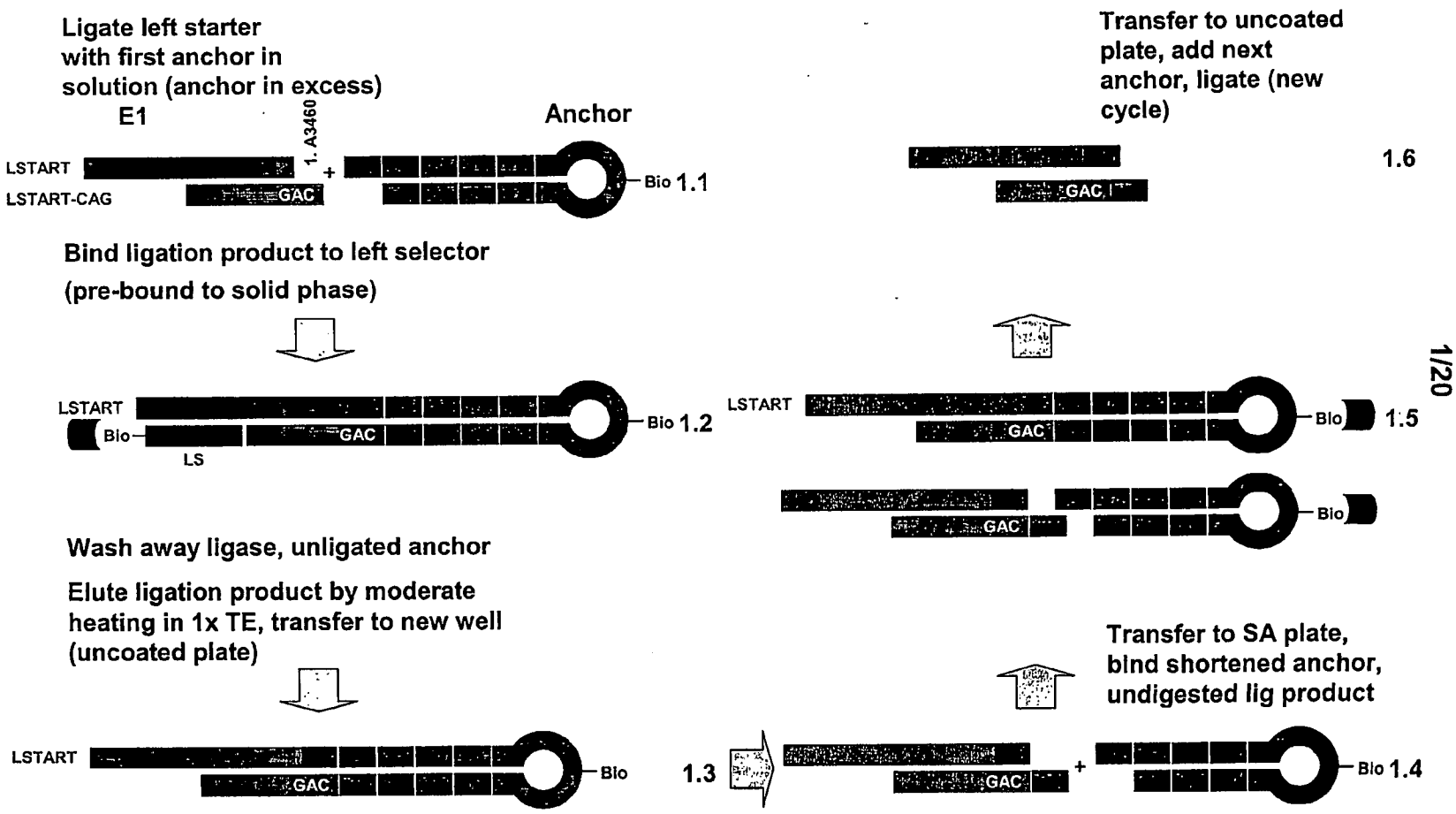


Fig. 2 – Structure of a double-selectable first order transposition product and its elongation block precursors

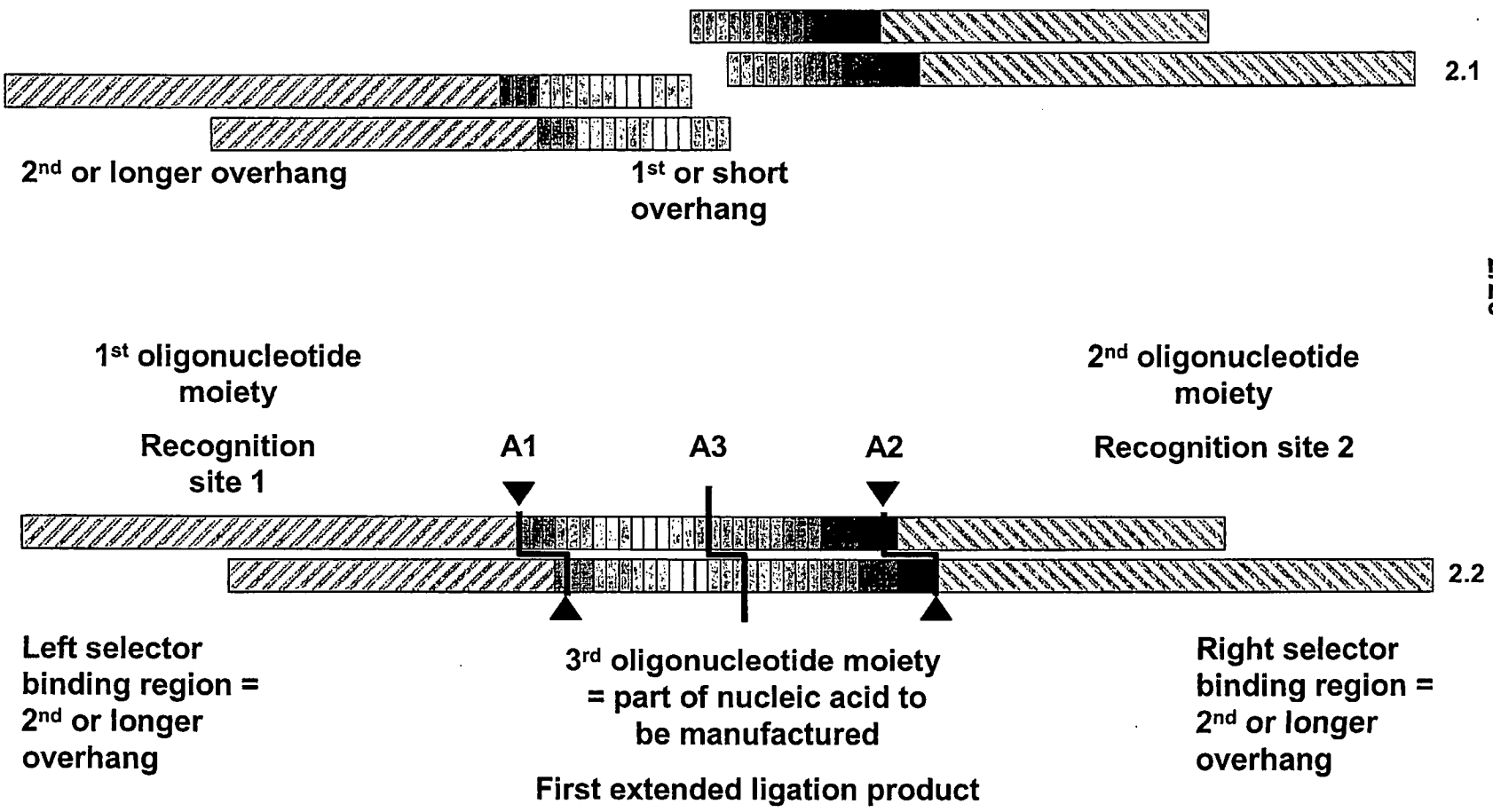
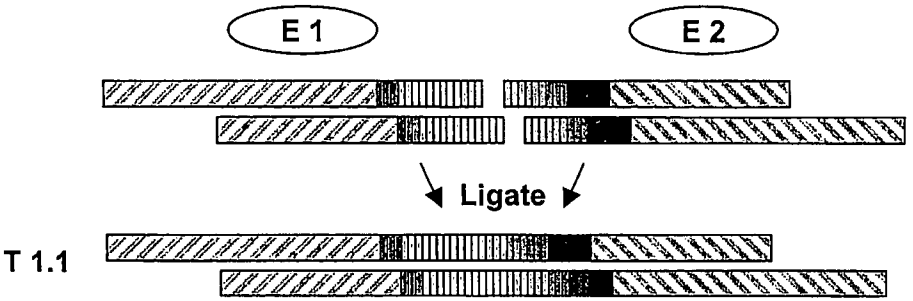


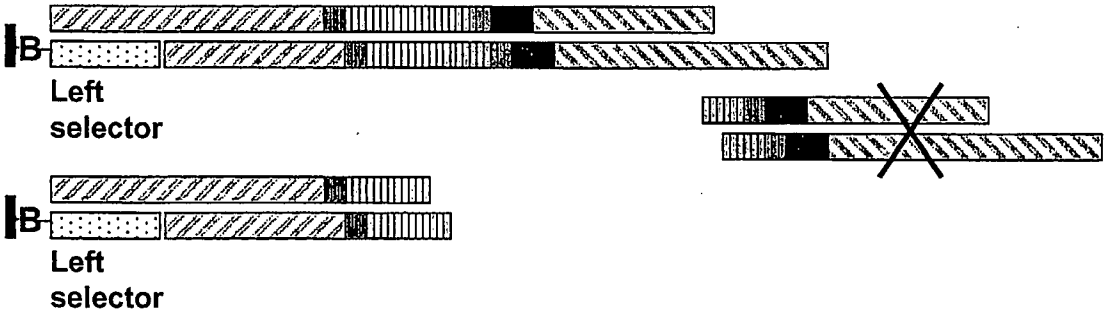
Fig. 3 – Double Selection procedure (I)

1. Ligate adjacent elongation products via complementary first overlap (see fig. 2)



3.1

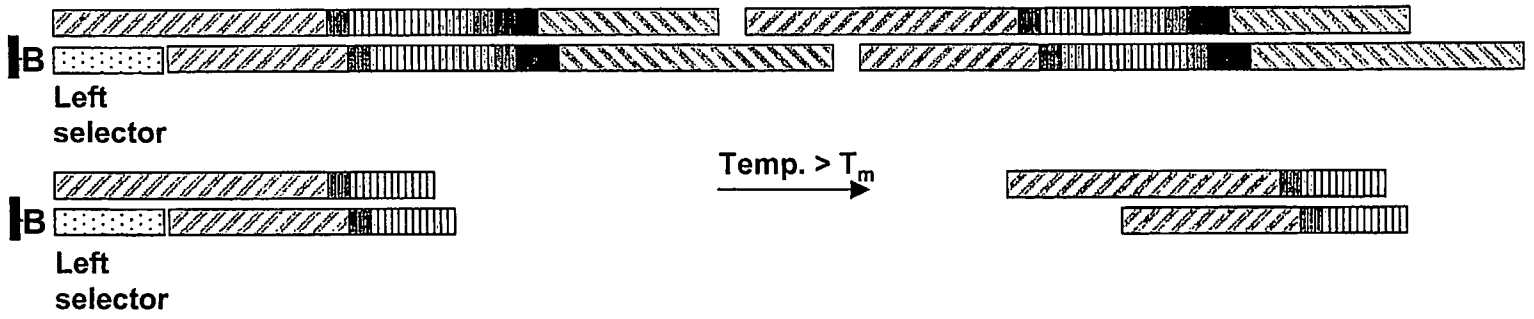
2. Anneal ligation product to left selector oligo (immobilised or in solution)
3. Bind to solid phase (if annealing was in solution)
4. Wash away any unreacted ligation partner containing right selector binding region only



3.2

Fig. 4 – Double Selection procedure (II)

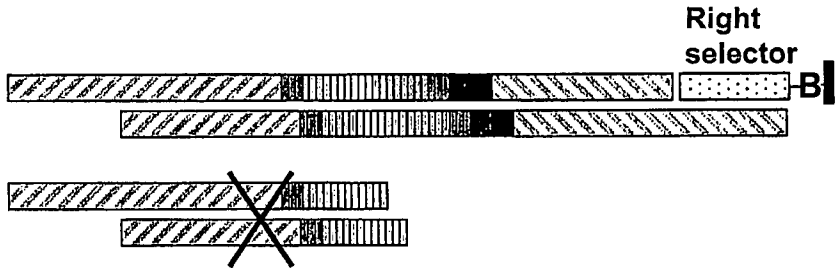
5. Elute ligation product and any remaining unreacted ligation partner containing left selector binding region only by heating beyond T_m of left selector hybrid



4.1

4.2

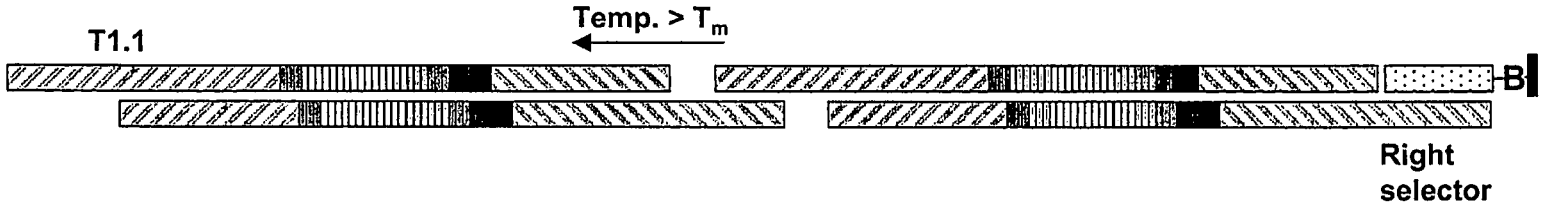
6. Anneal elution products to right selector oligo (immobilized or in solution)
7. Bind to solid phase (if annealing was in solution)
8. Wash away any remaining unreacted ligation partner containing left selector binding region only



4.3

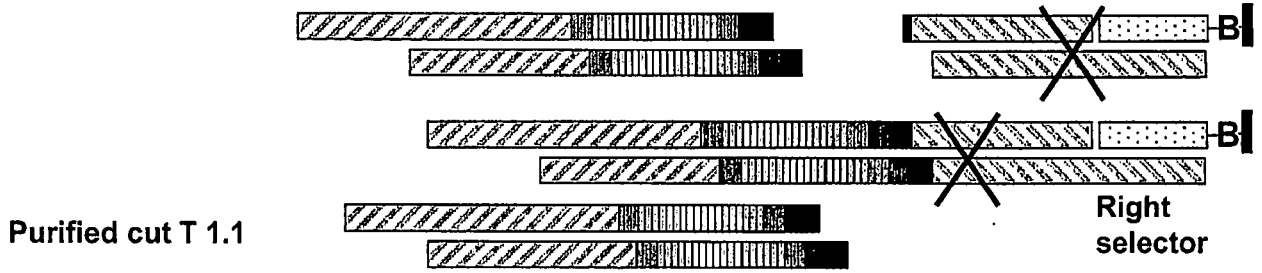
Fig. 5 – Double Selection procedure (III)

9. Elute pure T1.1 ligation product by heating above T_m of right selector hybrid, transfer to new vessel



10. Cut with restriction enzyme specific for the oligonucleotide moiety containing the right selector binding region

11. Anneal with right selector oligo (immobilized or in solution)
12. Bind to solid phase (if annealing was in solution) to remove the cut-off oligonucleotide moiety containing the right selector binding region as well as any uncut ligation product



13. Transfer cut transposition product (in supernatant) to new vessel
14. Use cut transposition product for further ligations

Fig. 6 – S-HIT procedure (Esp-Eco)

Structure of a ligation product

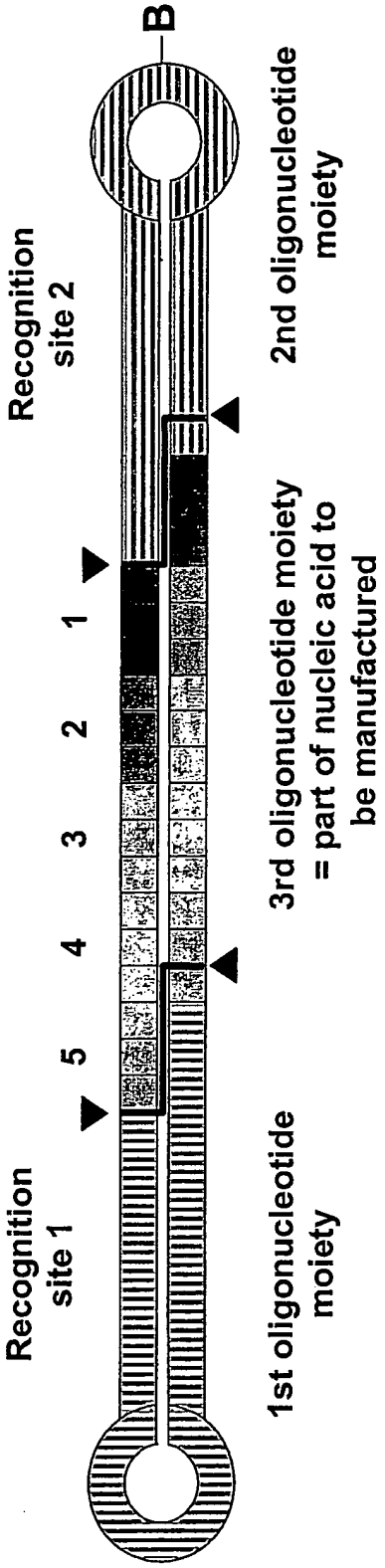


Fig. 7 – S-HIT procedure (Esp-Eco)

Elongation blocks E1 – E4 (arrows = orientation in target sequence)

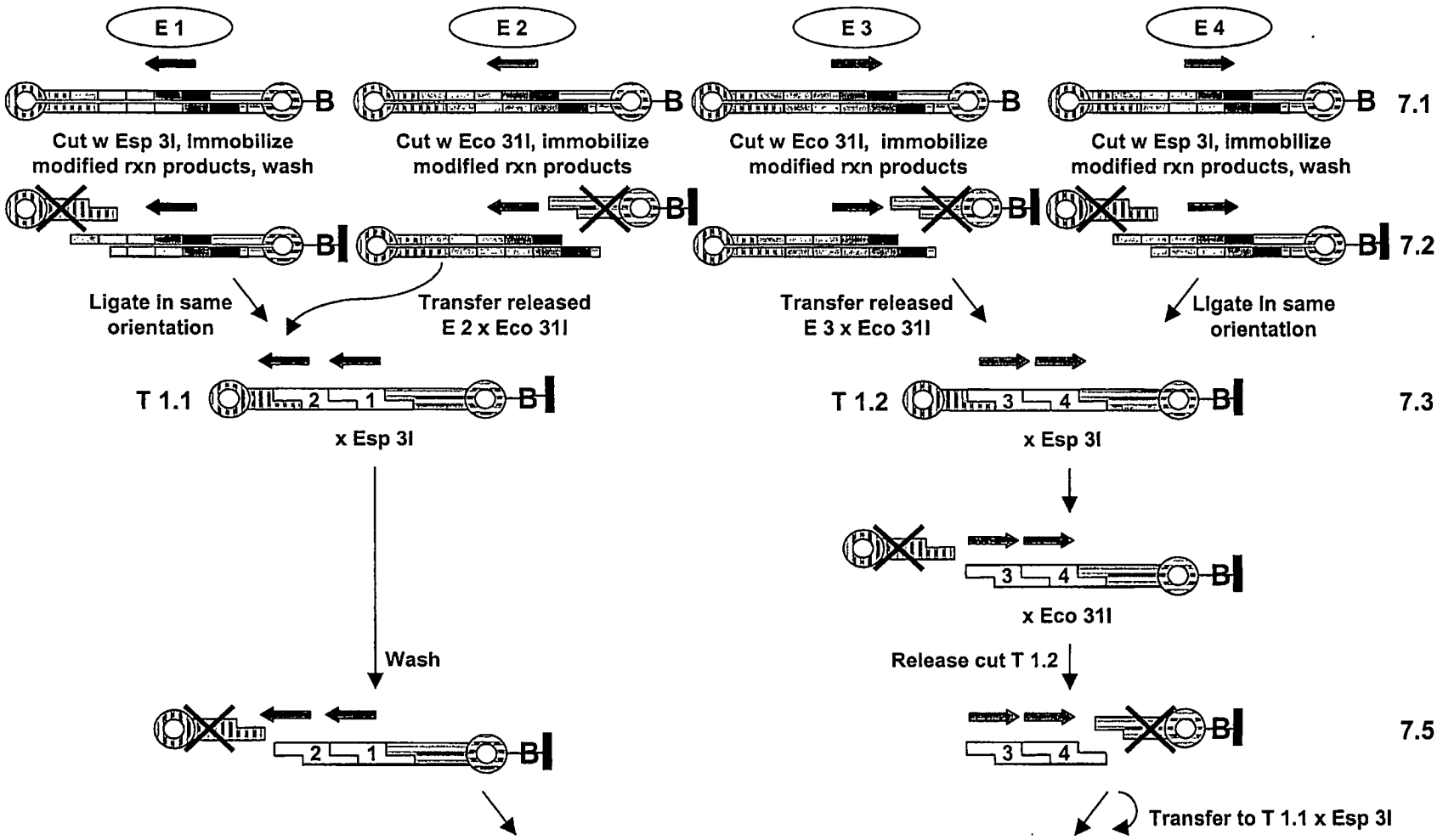


Fig. 8 – S-HIT procedure (Esp-Eco)

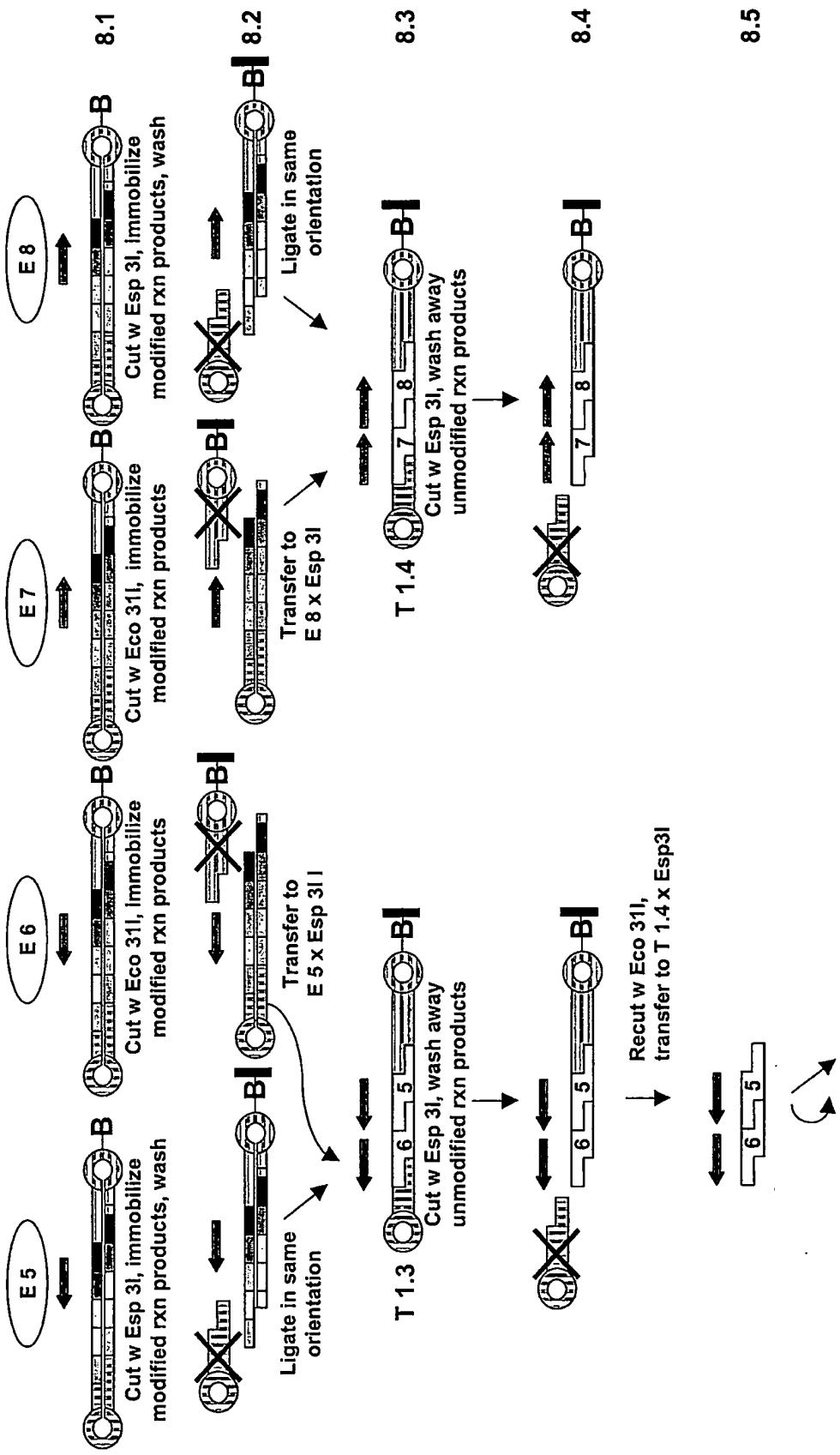


Fig. 9 – S-HIT procedure (Esp-Eco)

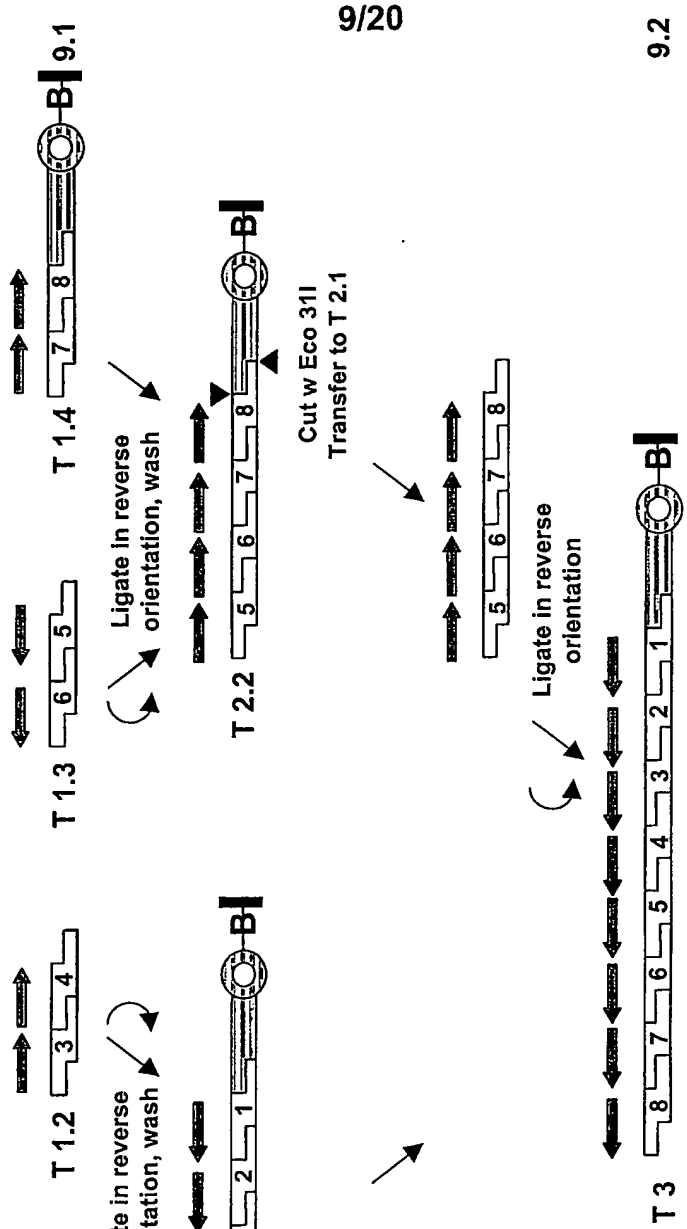


Fig. 10 – S-HIT procedure (Esp-Eam)

Structure of ligation products

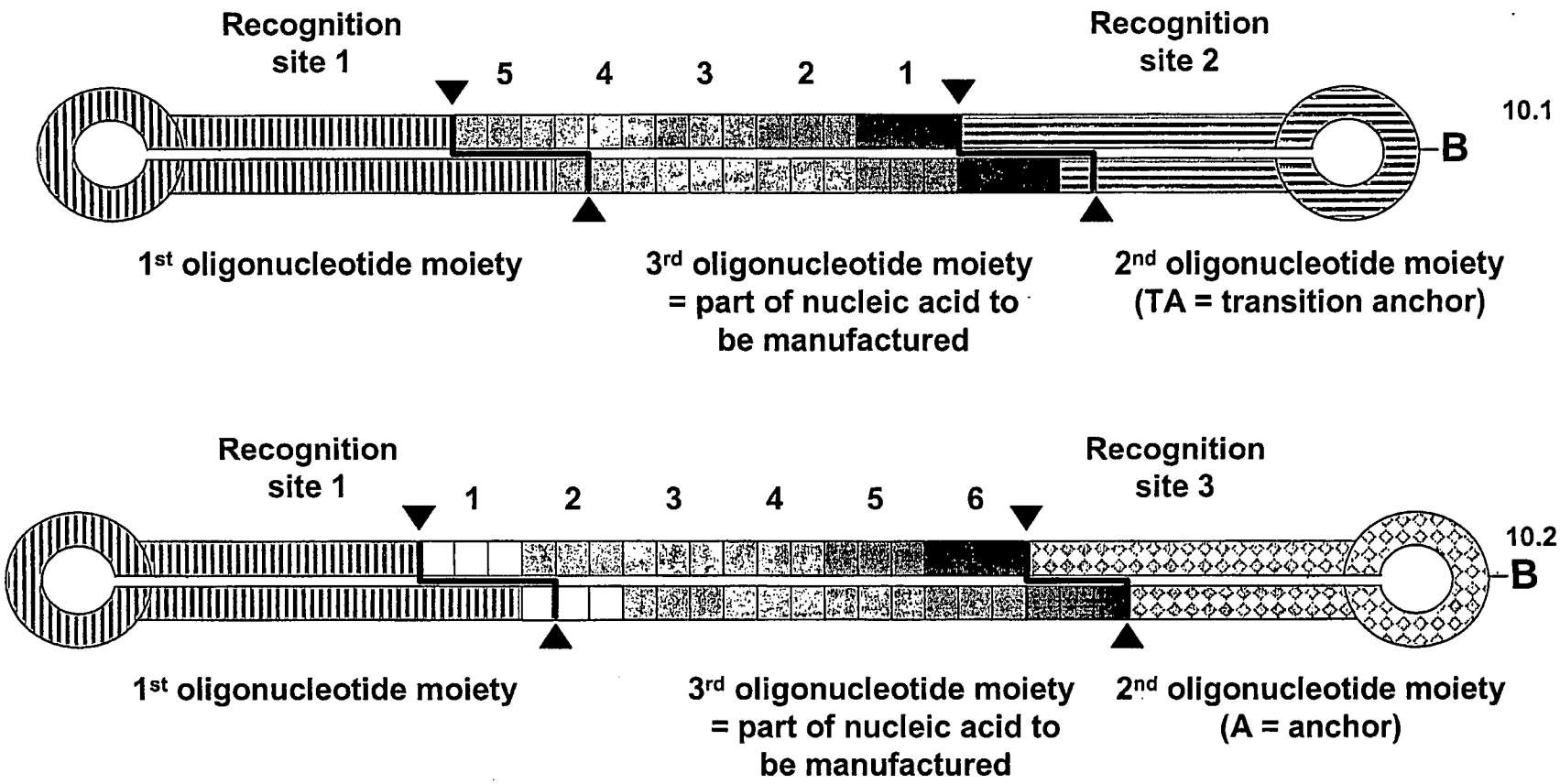


Fig. 11 – S-HIT procedure (Esp-Eam)

Elongation blocks E1 – E4 (arrows = orientation in target sequence)

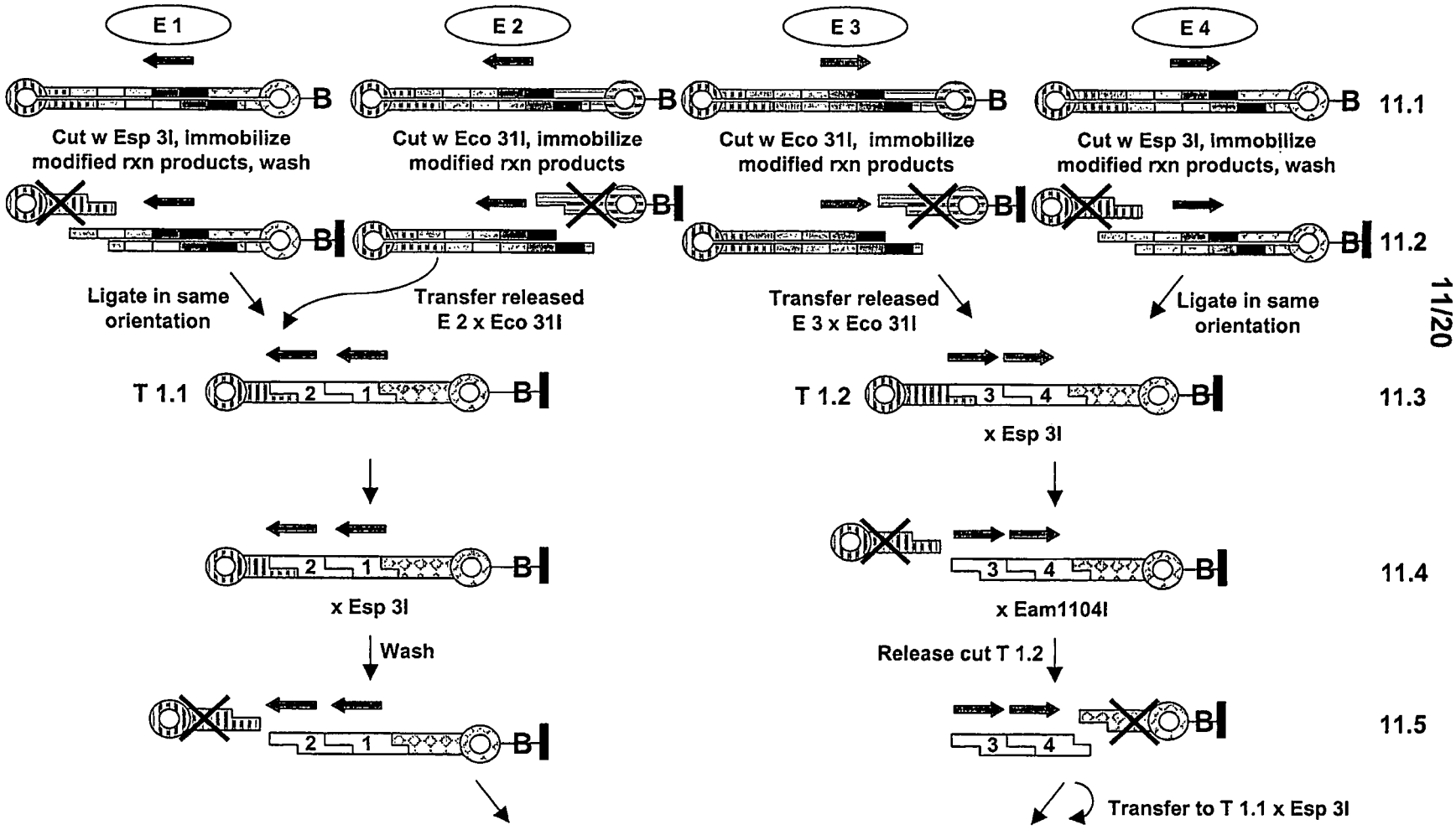


Fig. 12 – S-HIT procedure (Esp-Eam)

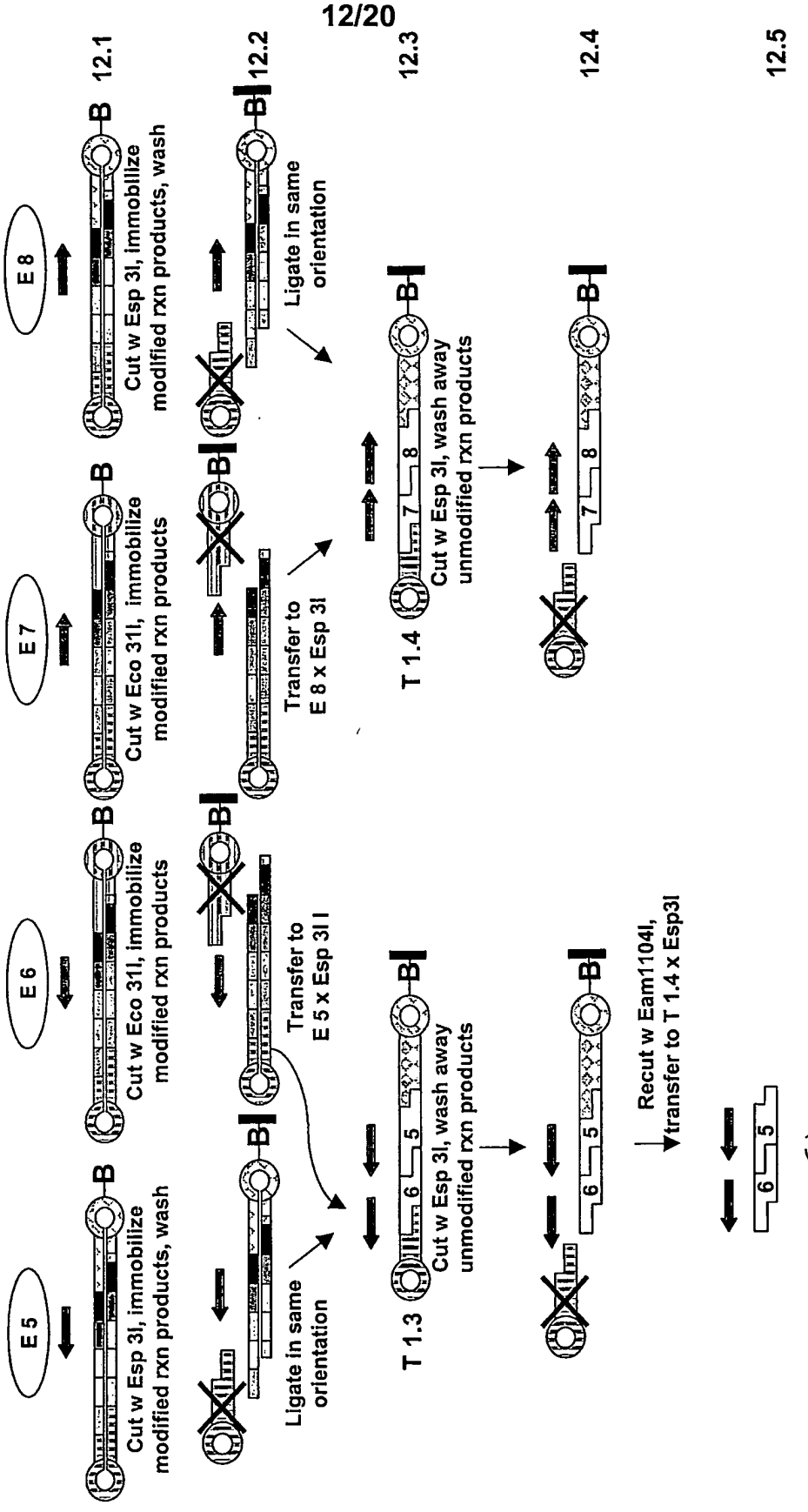


Fig. 13 – S-HIT procedure (Esp-Eam)

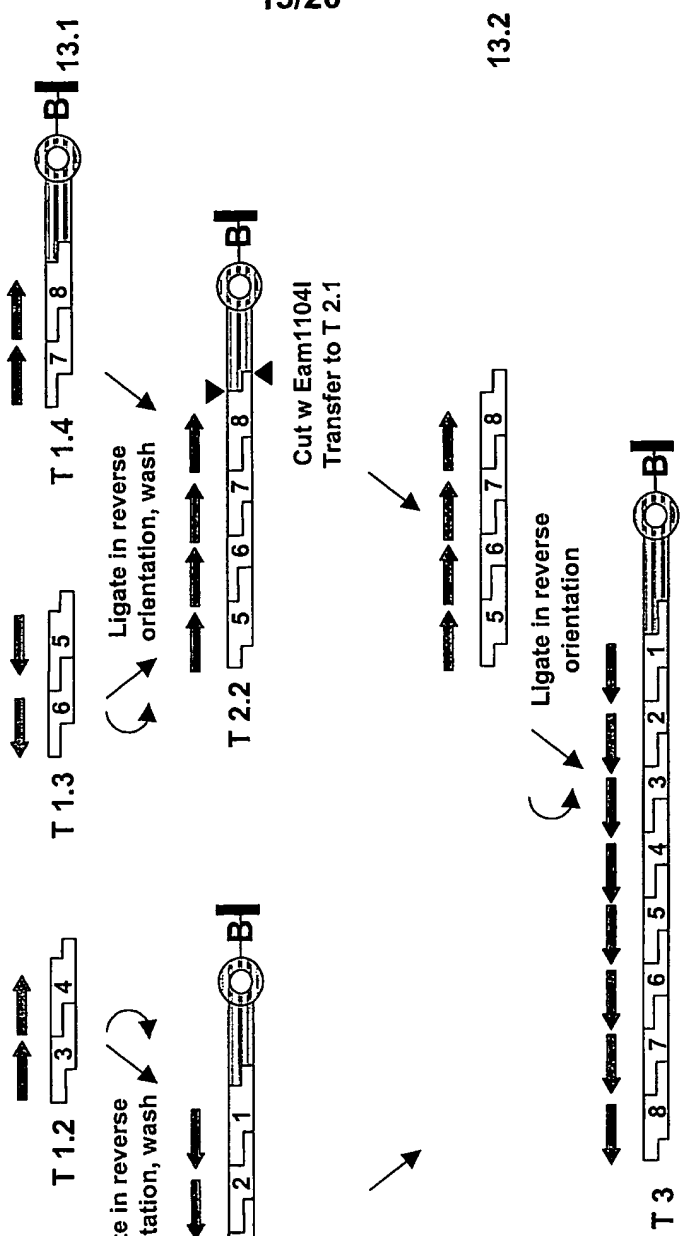


Fig. 14 – ASIT (Esp-Eco)

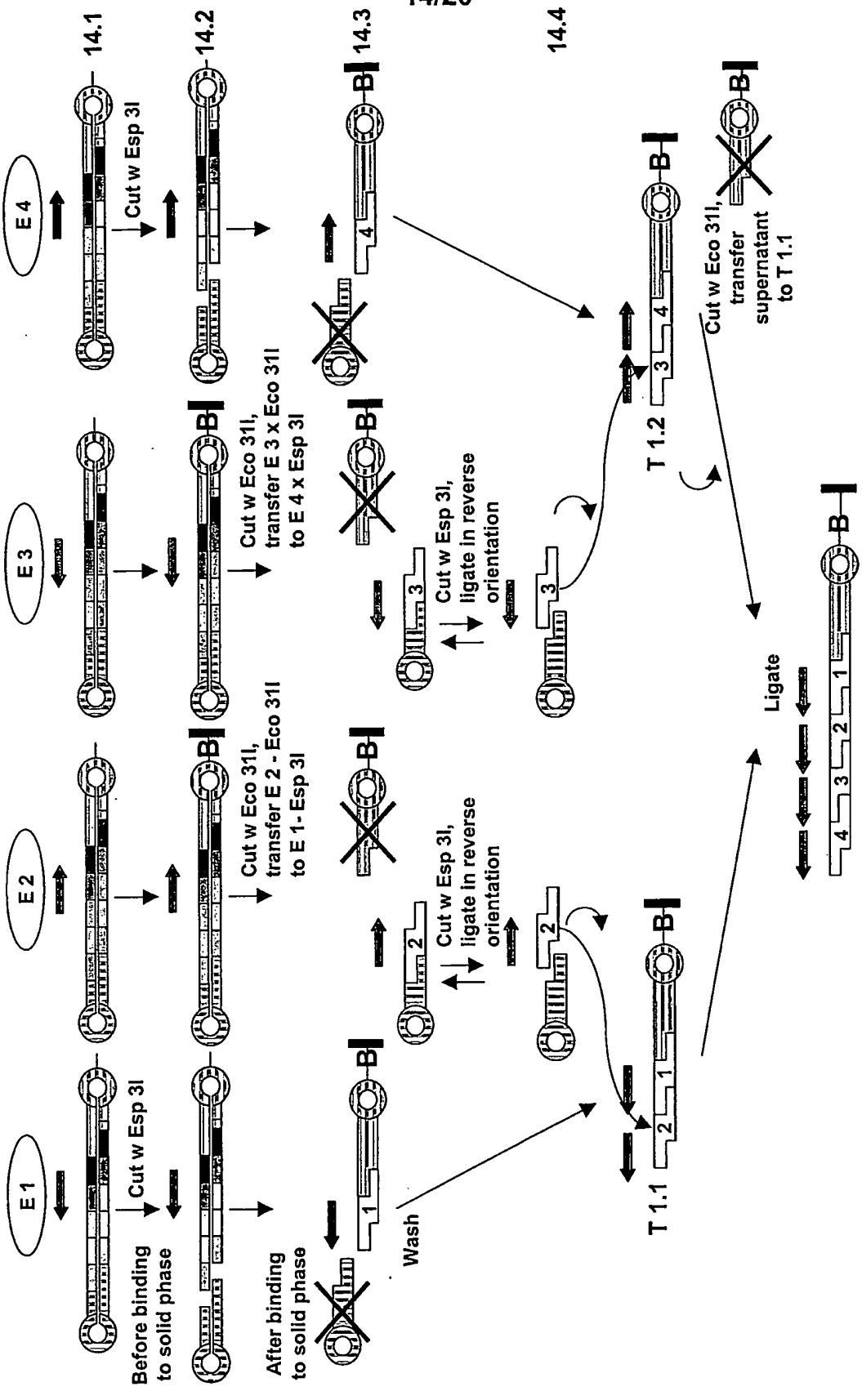


Fig. 15 – SIT (Esp-Eco)

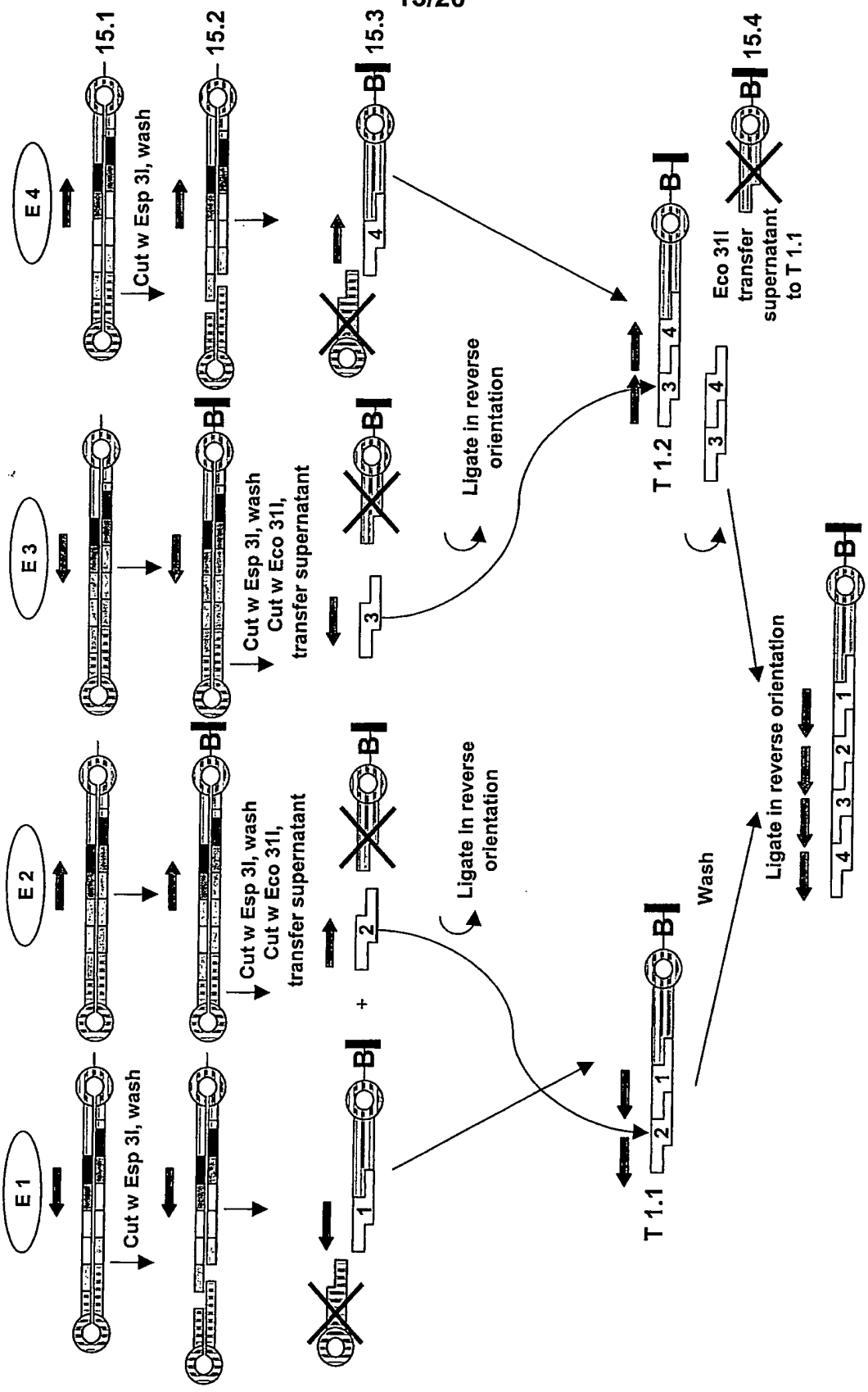


Fig. 16 – Capping oligonucleotide

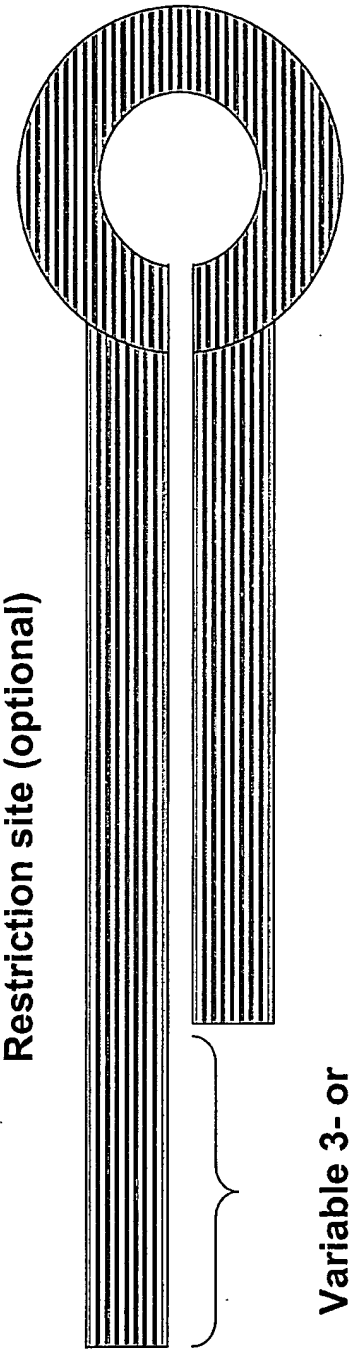


Fig. 17 – S4LS

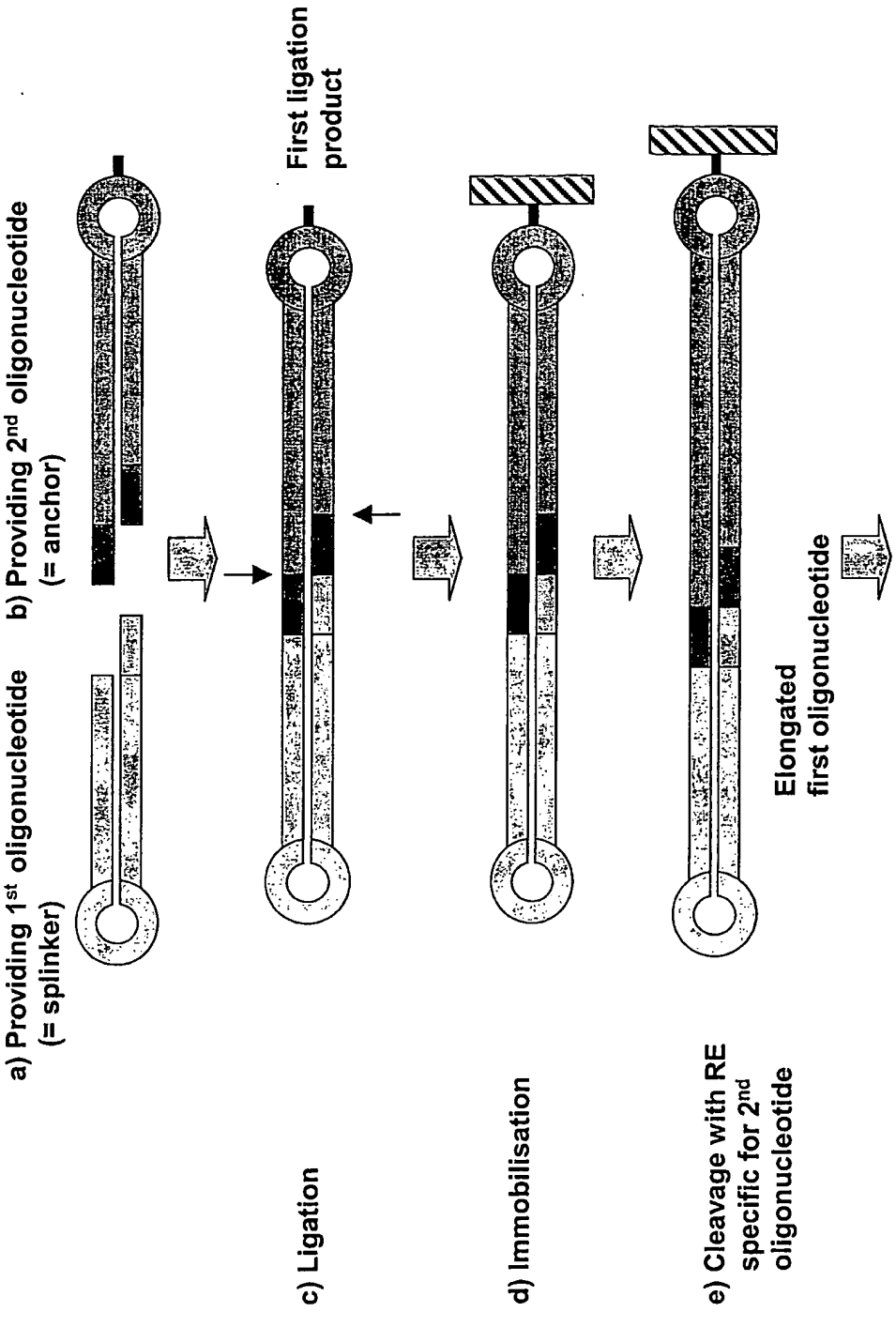

a) Providing 1st oligonucleotide (= splinker)
b) Providing 2nd oligonucleotide (= anchor)
First ligation product
c) Ligation
d) Immobilisation
e) Cleavage with RE specific for 2nd oligonucleotide
Elongated first oligonucleotide

Fig. 18 – S4LS

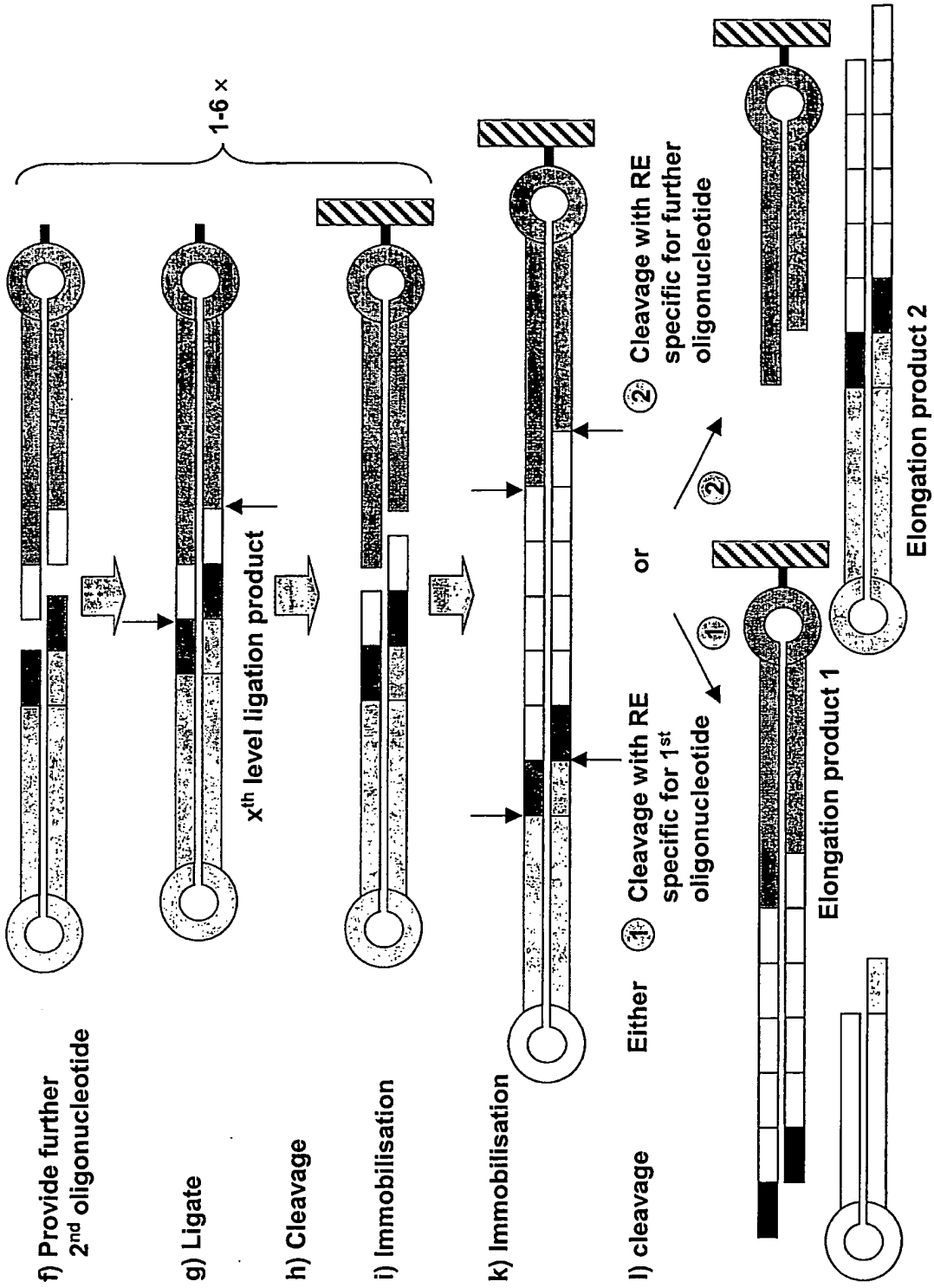

f) Provide further 2nd oligonucleotide
1-6 x
g) Ligate
xth level ligation product
h) Cleavage
i) Immobilisation
k) Immobilisation
l) cleavage
Either
1 Cleavage with RE specific for 1st oligonucleotide
or
2 Cleavage with RE specific for further oligonucleotide
Elongation product 1
Elongation product 2

Fig. 19 – S4LS vs RSPS

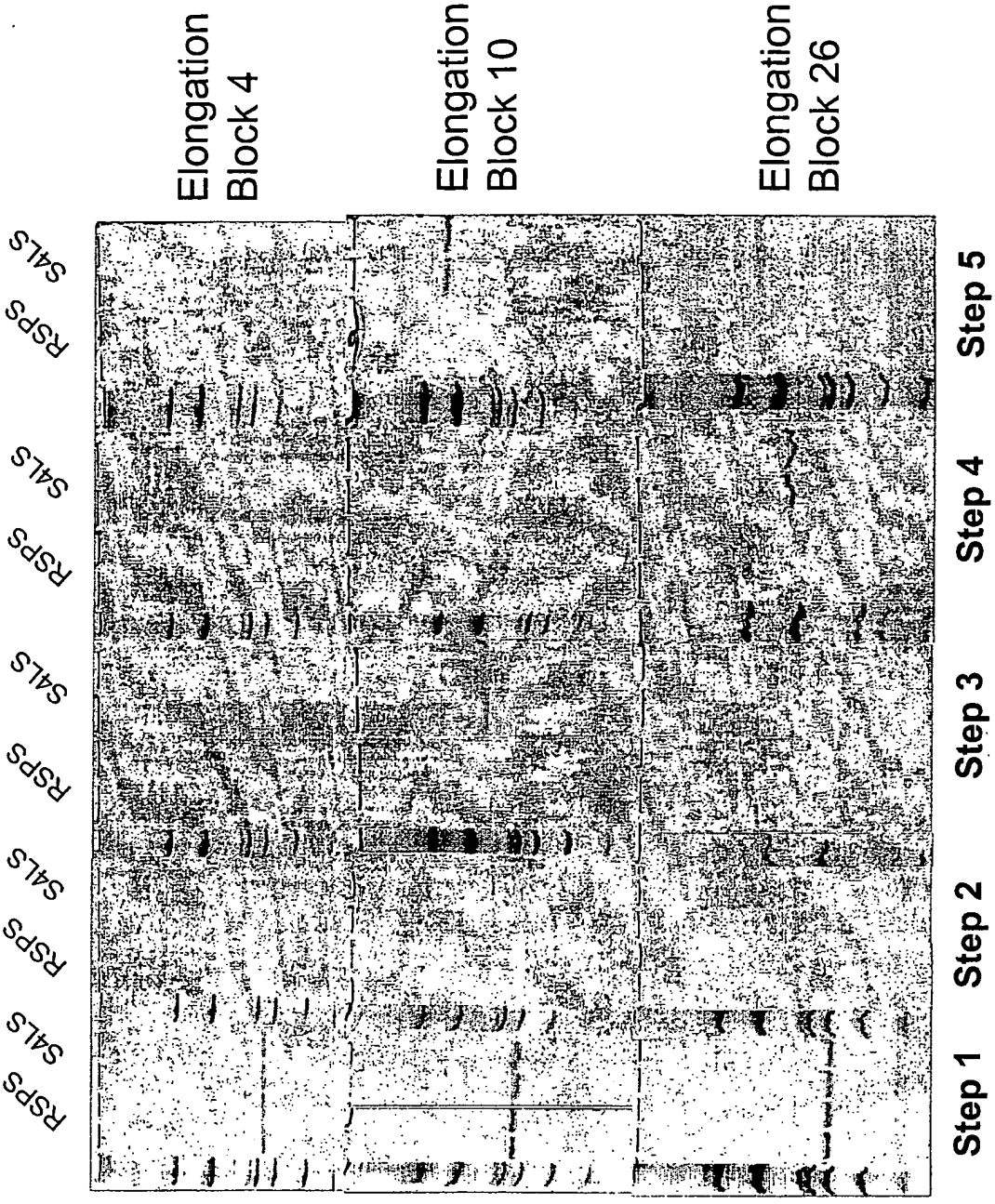


Fig. 20 – S4LS vs RSPS